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L3: Entry 16 of 23 File: USPT Nov 24, 1998

DOCUMENT-IDENTIFIER: US 5840900 A
TITLE: High molecular weight polymer-based prodrugs

Detailed Description Paragraph Right (1):
The prodrug compositions of the present invention contain hydrolyzable linkages between the polymer portion and a biologically active moiety derived from a biologically active moiety or nucleophile, i.e. native or unmodified drug. These linkages are preferably ester linkages designed to hydrolyze at a rate which generates sufficient amounts of the biologically active parent compound in a suitable time period so that therapeutic levels of the parent therapeutic moiety or moieties are delivered prior to excretion from or inactivation by the body. The term "sufficient amounts" for purposes of the present invention shall mean an amount which achieves a therapeutic effect as such effect is understood by those of ordinary skill in the art.

Detailed Description Paragraph Right (13):
In particular, polyethylene glycols (PEG's), mono-activated, C.sub.1-4 alkyl-terminated PAO's such as mono-methyl-terminated polyethylene glycols (mPEG's) are preferred when mono-substituted polymers are desired; bis-activated polyethylene oxides are preferred when disubstituted prodrugs are desired. In order to provide the desired hydrolyzable linkage, mono-or di-acid activated polymers such as PEG acids or PEG diacids are used. Suitable PAO acids can be synthesized by converting mPEG--OH to an ethyl ester. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F. M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG--OH into a t-butyl ester. See, for example, commonly assigned U.S. patent application Ser. No. 08/440,732 filed May 15, 1995 now U.S. Pat. No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.

Detailed Description Paragraph Right (30):
The only limitation on the types of molecules suitable for inclusion herein is that there is at least one position on which the hydrolyzable linkage can be attached, so that after prodrug administration, the prodrug can regenerate sufficient quantities of the parent compound in vivo.

Detailed Description Paragraph Right (33):
Examples of suitable bifunctional spacer groups include diglycolic acid, thiodiglycolic acid, l-alanine and d-alanine.

Detailed Description Paragraph Type 1 (12):
1) providing an activated polymer, such as a PEG-acid or PEG-diacid and a parent compound having a position thereon which will allow a hydrolyzable linkage to form, and

Detailed Description Paragraph Type 1 (14):
reacting a biologically active moiety containing an available hydroxyl group with a bifunctional spacer moiety containing an available carboxylic acid group in the presence of a first coupling agent to form a biologically active moiety--spacer prodrug intermediate,

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L3: Entry 18 of 23 File: USPT Jul 15, 1997

DOCUMENT-IDENTIFIER: US 5648506 A
TITLE: Water-soluble polymeric carriers for drug delivery

Detailed Description Paragraph Right (5):
Another approach used was to deliver taxol in a soluble form such as the PEG derivative that could be hydrolyzed to release taxol in an active form after delivery of the drug-polymer conjugate. In this method taxol was linked to PEG at the 2' position by a readily hydrolyzable ester linkage. Two approaches were adopted to synthesize this derivative. The first involved modifying the hydroxyl end groups of MPEG with succinic anhydride to obtain the succinyl derivative of MPEG (15). This derivative was esterified with the 2'-hydroxyl on taxol using dicyclohexyl carbodiimide (DCC) and 4-dimethylamino pyridine (DMAP) to obtain the derivative 7. The second approach involved modification of the 2'hydroxyl on taxol with succinic or glutaric anhydride to obtain the succinyl (8) or glutaryl (9) derivative of taxol which was esterified with the MPEG hydroxyl using DCC and DMAP as before. Both these procedures resulted in the formation of 2'-MPEG taxol (10 or 11) that was readily hydrolyzable in an aqueous environment to give back active taxol and water-soluble carrier. A monofunctional PEG (MPEG) or a bifunctional PEG (regular PEG) could be used for this reaction. PEG (MPEG and/or PEG) molecular weights 200-100000 (n=5-2500) could be utilized for the derivatization. A preferred range is 600-20000 (n=10-500) and the most preferred range is 1000-10000 (n=20-250).

Detailed Description Paragraph Right (23):
The above text describes the production of taxol derivatives with water-soluble polymers. These soluble polymeric carriers containing the bound drug may be crosslinked to produce an insoluble polymer matrix which is water-swellable and has hydrogel properties. Such a matrix may be prepared in the form of a sphere, disc, cylinder, etc. that could be subsequently implanted at a suitable site for sustained release of the bound drug by hydrolysis. Such a matrix is prepared by utilizing a branched or star PEG in which a portion of the available sites are functionalized by polymerizable acrylate or methacrylate groups and the remainder are bound to the drug. This polymer is isolated, dissolved in aqueous buffer (or organic solvent) and crosslinked by a free radical process that may be thermally initiated or photoinitiated. Following crosslinking, the gel is dessicated by drying in vacuum and stored dry until before use when it is hydrated. To carry out the crosslinking step in organic solvent, the polymer is dissolved at a suitable concentration to obtain a solution of mild viscosity, a thermal initiator such as AIBN, or a UV photoinitiator such as 2,2-dimethoxy-2-phenyl acetophenone (DMPA) is added. To prepare the gel in the form of a disk, the solution is poured into a mould and heated or exposed to long wave UV radiation to crosslink the polymer. If the crosslinking step is to be carried out in aqueous medium, the same proceedure is followed except for replacing the organic solvent with an aqueous buffer, adding a water-soluble UV initiator such as 2,2'-azobis-(2-amidinopropane)hydrochloride (AAPH) and exposing to UV light, or using a visible light initiated system comprising the dye ethyl eosin and cocatalyst triethanol amine and exposing the sample to green light in the region of 500-580 nm. A small quantity of bifunctional crosslinkers may also be added, e.g., tetraethylene glycol diacrylate. The degree of substitution of available sites by polymerizable groups is varied depending on the degree of crosslinking and drug loading required. The presently preferred ratio of unsaturated groups to all available sites is between 0.04 and 0.75. A more preferred range is between 0.1 to 0.5.

Detailed Description Paragraph Right (33):
Water-soluble Prodrugs of Taxol: PEG at the 2' Position Bound by a Hydrolyzable Linkage (Reaction of Taxol 2'-OH with PEG-COOH)

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L8: Entry 1 of 2 File: USPT Jan 29, 2002

DOCUMENT-IDENTIFIER: US 6342221 B1
TITLE: Antibody conjugate compositions for selectively inhibiting VEGF

Brief Summary Paragraph Right (13):
The recognition of VEGF as a primary stimulus of angiogenesis in pathological conditions has led to various attempts to block VEGF activity. Inhibitory anti-VEGF receptor antibodies, soluble receptor constructs, antisense strategies, RNA aptamers against VEGF and low molecular weight VEGF receptor tyrosine kinase (RTK) inhibitors have all been proposed for use in interfering with VEGF signaling (Siemeister et al., 1998). In fact, monoclonal antibodies against VEGF have been shown to inhibit human tumor xenograft growth and ascites formation in mice (Kim et al., 1993; Asano et al., 1998; Mesiano et al., 1998; Luo et al., 1998a; 1998b; Borgstrom et al., 1996; 1998).

Detailed Description Paragraph Right (26):
The application of various inhibitory methods has been shown to be at least somewhat effective in either blocking angiogenesis and/or suppressing tumor growth by interfering with VEGF signaling. In fact, monoclonal antibodies against VEGF have been shown to inhibit human tumor xenograft growth and ascites formation in mice (Kim et al., 1993; Asano et al., 1995; 1998; Mesiano et al., 1998; Luo et al., 1998a; 1998b; Borgstrom et al., 1996; 1998).

Detailed Description Paragraph Right (59):
The magnitude of tumor growth suppression by 2C3 is similar to that reported by other investigators using different neutralizing anti-VEGF antibodies (Asano et al., 1998; Mesiano et al., 1998). A monoclonal rat anti-mouse VEGFR2 antibody also strongly blocked the growth of malignant human keratinocytes in mice through an anti-angiogenic mechanism (Skobe et al., 1997). The effectiveness of 2C3, being similar to what other investigators have found using different anti-VEGF antibodies, further demonstrates the role of VEGF in tumor angiogenesis and tumor growth. However, 2C3 should provide a safer therapeutic, based on the specific inhibitory properties discussed herein.

Detailed Description Paragraph Right (199):
The usual procedure for preparation of F(ab').sub.2 fragments from IgG of rabbit and human origin is limited proteolysis by the enzyme pepsin. The conditions, 100.times.antibody excess w/w in acetate buffer at pH 4.5, 37.degree. C., suggest that antibody is cleaved at the C-terminal side of the inter-heavy-chain disulfide bond. Rates of digestion of mouse IgG may vary with subclass and conditions should be chosen to avoid significant amounts of completely degraded IgG. In particular, IgG.sub.2b is susceptible to complete degradation. The other subclasses require different incubation conditions to produce optimal results, all of which is known in the art.

Detailed Description Paragraph Right (316):
In additional to the general information provided above, VEGFR2-blocking, anti-VEGF antibody or 2C3-based antibodies may be conjugated to one or more therapeutic agents using certain preferred biochemical cross-linkers. Cross-linking reagents are used to form molecular bridges that tie together functional groups of two different molecules. To link two different proteins in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation. Exemplary hetero-bifunctional cross-linkers are referenced in Table B1.

Detailed Description Paragraph Right (317):
Hetero-bifunctional cross-linkers contain two reactive groups: one generally

reacting with primary amine group (e.g., N-hydroxy succinimide) and the other generally reacting with a thiol group (e.g., pyridyl disulfide, maleimides, halogens, etc.). Through the primary amine reactive group, the cross-linker may react with the lysine residue(s) of one protein (e.g., the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (e.g., the coagulant).

Detailed Description Paragraph Right (321):
One of the most preferred cross-linking reagents for use in immunotoxins is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is "sterically hindered" by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the tumor site. It is contemplated that the SMPT agent may also be used in connection with the bispecific ligands of this invention.

Detailed Description Paragraph Right (322):
The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (e.g., the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

Detailed Description Paragraph Right (363):
Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate, which matrices are in the form of shaped articles, e.g., films or microcapsule. Examples of sustained-release matrices include polyesters; hydrogels, for example, poly(2-hydroxyethyl-methacrylate) or poly(vinylalcohol); polylactides, e.g. , U.S. Pat. No. 3,773,919; copolymers of L-glutamic acid and .gamma. ethyl-L-glutamate; non-degradable ethylene-vinyl acetate; degradable lactic acid-glycolic acid copolymers, such as the Lupron Depot.TM. (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate); and poly-D-(-)-3-hydroxybutyric acid.

Detailed Description Paragraph Right (370):
Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 .mu.m) should be designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be are easily made.

Detailed Description Paragraph Right (482):
Tissue inhibitors of metalloproteinases (TIMPs) are a family of naturally occurring inhibitors of matrix metalloproteases (MMPs) that can also inhibit angiogenesis and can be used in combined treatment protocols. MMPs play a key role in the angiogenic process as they degrade the matrix through which endothelial cells and fibroblasts migrate when extending or remodeling the vascular network. In fact, one member of the MMPs, MMP-2, has been shown to associate with activated endothelium through the integrin .alpha.v.beta.3 presumably for this purpose. If this interaction is disrupted by a fragment of MMP-2, then angiogenesis is downregulated and in tumors growth is inhibited.

Detailed Description Paragraph Right (659):
Mice bearing subcutaneous NCI-H358 NSCLC tumors that had grown to a size of approximately 300-450 mm.sup.3 were injected i.p. with 2C3, A4.6.1, 3E7, or an IgG of irrelevant specificity (FIG. 4). Doses were 50-100 .mu.g every 3-5 days. A4.6.1 was used as a positive control because it has been shown by other investigators to block VEGF activity in vivo resulting in an inhibition of tumor growth (Kim et al., 1993; Mesiano et al., 1998). In addition to measuring mean tumor volume (FIG. 4), photographs of the mice from each treatment group were also taken to show the differences in tumor size and appearance at the end of the study.

Detailed Description Paragraph Right (694):
.beta.-glucuronide prodrugs, such as doxorubicin-.beta.-glucuronide and calcimycin-.beta.-glucuronide were prepared essentially as described in U.S. Pat. No. 5,561,119, specifically incorporated herein by reference. Such prodrugs are designed to release the cytotoxic component, such as doxorubicin or calcimycin, only when degraded by a glycoside enzyme, such as GUS. By attaching GUS to 2C3, GUS is targeted specifically to the tumor vasculature and stroma, thus providing for specific cleavage of the prodrugs and release of the cytotoxic component specifically within the tumor site.

Detailed Description Paragraph Type 0 (44):
Davis and Yancopoulos, "The angiopoietins: Yin and Yang in angiogenesis", Curr. Top. Microbiol. Immunol., 237:173-85, 1999.

Detailed Description Paragraph Type 0 (139):
Mesiano, Ferrara, Jaffe, "Role of vascular endothelial growth factor in ovarian cancer: inhibition of ascites formation by immunoneutralization," Am. J. Pathol., 153(4):1249-1256, 1998.

Detailed Description Paragraph Table (2):
TABLE B1 HETERO-BIFUNCTIONAL CROSS-LINKERS Spacer Arm Length Linker Reactive Toward Advantages and Applications after cross-linking SMPT Primary amines Greater stability 11.2 A Sulfhydryls SPDP Primary amines Thiolation 6.8 A Sulfhydryls Cleavable cross-linking LC-SPDP Primary amines Extended spacer arm 15.6 A Sulfhydryls Sulfo-LC-SPDP Primary amines Extended spacer arm 15.6 A Sulfhydryls Water-soluble SMCC Primary amines Stable maleimide reactive group 11.6 A Sulfhydryls Enzyme-antibody conjugation Hapten-carrier protein conjugation Sulfo-SMCC Primary amines Stable maleimide reactive group 11.6 A Sulfhydryls Water-soluble Enzyme-antibody conjugation MBS Primary amines Enzyme-antibody conjugation 9.9 A Sulfhydryls Hapten-carrier protein conjugation Sulfo-MBS Primary amines Water-soluble 9.9 A Sulfhydryls SIAB Primary amines Enzyme-antibody conjugation 10.6 A Sulfhydryls Sulfo-SIAB Primary amines Water-soluble 10.6 A Sulfhydryls SMPB Primary amines Extended spacer arm 14.5 A Sulfhydryls Enzyme-antibody conjugation Sulfo-SMPB Primary amines Extended spacer arm 14.5 A Sulfhydryls Water-soluble EDC/Sulfo-NHS Primary amines Hapten-Carrier conjugation 0 Carboxyl groups ABH Carbohydrates Reacts with sugar groups 11.9 A Nonselective

Other Reference Publication (28):
Davis and Yancopoulos, "The Angiopoietins: Yin and Yang in Angiogenesis", Curr. Top. Microbiol. Immunol., 237:173-85, 1999.

Other Reference Publication (50):
Mesiano, et al., "Role of Vascular Endothelial Growth Factor in Ovarian Cancer: Inhibition of Ascites Formation by Immunoneutralization," Am. J. Pathol., 153(4):1249-1256, 1998.

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L11: Entry 2 of 11 File: USPT Jan 29, 2002

DOCUMENT-IDENTIFIER: US 6342221 B1
TITLE: Antibody conjugate compositions for selectively inhibiting VEGF

Brief Summary Paragraph Right (180):
Immunoconjugates with radiotherapeutic agents, anti-angiogenic agents, apoptosis-inducing agents, anti-tubulin drugs, toxins and coagulants, whether prepared by chemical conjugation or recombinant expression, may employ a biologically-releasable bond and/or a selectively cleavable spacer or linker. Such compositions are preferably reasonably stable during circulation and are preferentially or specifically released upon delivery to the disease or tumor site.

Detailed Description Paragraph Right (316):
In additional to the general information provided above, VEGFR2-blocking, anti-VEGF antibody or 2C3-based antibodies may be conjugated to one or more therapeutic agents using certain preferred biochemical cross-linkers. Cross-linking reagents are used to form molecular bridges that tie together functional groups of two different molecules. To link two different proteins in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation. Exemplary hetero-bifunctional cross-linkers are referenced in Table B1.

Detailed Description Paragraph Right (317):
Hetero-bifunctional cross-linkers contain two reactive groups: one generally reacting with primary amine group (e.g., N-hydroxy succinimide) and the other generally reacting with a thiol group (e.g., pyridyl disulfide, maleimides, halogens, etc.). Through the primary amine reactive group, the cross-linker may react with the lysine residue(s) of one protein (e.g., the selected antibody or fragment) and through the thiol reactive group, the cross-linker, already tied up to the first protein, reacts with the cysteine residue (free sulfhydryl group) of the other protein (e.g., the coagulant).

Detailed Description Paragraph Right (321):
One of the most preferred cross-linking reagents for use in immunotoxins is SMPT, which is a bifunctional cross-linker containing a disulfide bond that is "sterically hindered" by an adjacent benzene ring and methyl groups. It is believed that steric hindrance of the disulfide bond serves a function of protecting the bond from attack by thiolate anions such as glutathione which can be present in tissues and blood, and thereby help in preventing decoupling of the conjugate prior to the delivery of the attached agent to the tumor site. It is contemplated that the SMPT agent may also be used in connection with the bispecific ligands of this invention.

Detailed Description Paragraph Right (322):
The SMPT cross-linking reagent, as with many other known cross-linking reagents, lends the ability to cross-link functional groups such as the SH of cysteine or primary amines (e.g., the epsilon amino group of lysine). Another possible type of cross-linker includes the hetero-bifunctional photoreactive phenylazides containing a cleavable disulfide bond such as sulfosuccinimidyl-2-(p-azido salicylamido) ethyl-1,3'-dithiopropionate. The N-hydroxy-succinimidyl group reacts with primary amino groups and the phenylazide (upon photolysis) reacts non-selectively with any amino acid residue.

Detailed Description Paragraph Right (326):
Although it is preferred that any linking moiety will have reasonable stability in blood, to prevent substantial release of the attached agent before targeting to the disease or tumor site, in certain aspects, the use of biologically-releasable bonds and/or selectively cleavable spacers or linkers is contemplated.

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L3: Entry 8 of 23 File: USPT Jun 26, 2001

DOCUMENT-IDENTIFIER: US 6251382 B1
TITLE: Biodegradable high molecular weight polymeric linkers and their conjugates

Detailed Description Paragraph Right (10):
The M moiety provides the backbone for attaching the various polymeric and biologically active moieties. Those skilled in the art will readily appreciate that generally the M moiety is derived from a group which is at least bifunctional and includes at least two sites for drug or polymer attachment.

Detailed Description Paragraph Right (14):
In order to provide the desired hydrolyzable linkage, mono- or di-acid activated polymers such as PEG acids or PEG diacids can be used as well as mono- or di-PEG amines and mono- or di-PEG diols. Suitable PAO acids can be synthesized by first converting MPEG-OH to an ethyl ester followed by saponification. See also Gehrhardt, H., et al. Polymer Bulletin 18: 487 (1987) and Veronese, F. M., et al., J. Controlled Release 10; 145 (1989). Alternatively, the PAO-acid can be synthesized by converting mPEG-OH into a t-butyl ester followed by acid cleavage. See, for example, commonly signed U.S. Pat. No. 5,605,976. The disclosures of each of the foregoing are incorporated by reference herein.